Biomonitoring of Exposure to Polycyclic Aromatic Hydrocarbons of Nonoccupationally Exposed Persons[1]

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Abstract

In a field study with 69 subjects, we investigated the influence of smoking, exposure to environmental tobacco smoke (ETS), diet, and location of residence on biomarkers for polycyclic aromatic hydrocarbons (PAH), including urinary excretion of 1-hydroxypyrene and benzo[*a*]pyrene (BaP) adducts of hemoglobin and albumin. The self-reported smoking status and the extent of ETS exposure were verified by urinary cotinine measurements. ETS exposure was quantified by nicotine and 3-ethenylpyridine measurements on personal samplers worn by the nonsmokers over 5 or 7 days before blood and urine samples were collected. Smokers ($n = 27$), on average, excreted 0.346 μg/24 h 1-hydroxypyrene, whereas the corresponding value for nonsmokers ($n = 42$) was 0.157 μg/24 h. Average BaP adduct levels with hemoglobin and albumin were 0.105 fmol/mg and 0.042 fmol/mg, respectively, for smokers, and 0.068 fmol/mg and 0.020 fmol/mg, respectively, for nonsmokers. The differences, except for the hemoglobin adducts, were statistically significant. Of the 42 nonsmokers, 19 were classified as passive smokers. There was no significant difference in the PAH biomarkers between nonsmokers exposed to ETS and those not or rarely exposed to ETS.

Total dietary BaP intake, as calculated from questionnaire data, did not correlate with any of the PAH biomarkers ($r < 0.1$). Subjects living in the suburbs tended to have higher BaP-protein adduct levels than subjects living in the city.

Our findings suggest that diet and smoking are major sources for PAH exposure of persons not occupationally exposed to PAH, whereas the influence of ETS exposure is negligible. The lack of correlation between the dietary PAH intake and the PAH biomarkers may be due to the inaccuracy of the estimate for the dietary PAH intake.

Introduction

PAHs[3] are formed during incomplete combustions and occur ubiquitously in the environment and at various workplaces (1). Eleven members of the class of PAH (*e.g.*, BaP) are carcinogenic (1). For nonoccupationally exposed persons, diet, ambient air, tobacco smoke, and coal-tar-containing medications are the main sources of PAH exposure (2). The daily intake of BaP with the diet is estimated to range from 120–2800 ng/day (2). Mainstream smoke yields of BaP for filter cigarettes amount to about 10 ng/cigarette (3, 4), leading to an intake of about 200 ng/day for a pack-a-day cigarette smoker. BaP intake from ambient air by inhalation and from water is predicted to contribute about 2% and 1%, respectively, to the total daily intake of BaP in nonsmokers (2). The contribution of ETS exposure is estimated to be <1% (5).

Biomonitoring is an especially valuable method to provide exposure data on multimedia environmental contaminants such as PAH (6, 7). Various urinary PAH metabolites such as 1-OHP, 1-OHP-glucuronide, monohydroxy-phenanthrenes, and BaP (after chemical reduction of the BaP metabolites in urine) have been used as PAH biomarkers in nonoccupationally exposed subjects. The effective dose can be determined by measuring the adducts formed by the ultimate carcinogen of BaP (r-7,t-8-dihydroxy-t-9,t-10-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene, BPDE) with cellular macromolecules such as DNA and proteins. In addition to numerous biomonitoring studies with workers exposed to PAH (for review see Refs. 7 and 8), the effect of smoking, diet, automobile traffic, and ETS on the PAH adduct levels has been investigated using DNA of WBCs. BPDE also forms covalent adducts with albumin (9) and hemoglobin (10), both of which can be used as surrogate biomarkers for the internal exposure dose to the ultimate carcinogen BPDE (11). BaP-albumin adduct studies with nonoccupationally exposed subjects have been performed using ELISA (12–15), GC-MS (16), and HPLC with fluorescence detection (17). Similarly, BaP hemoglobin adducts have been investigated with GC-MS (16, 18–20). Smoking was found to increase the BaP protein adduct levels in most of the studies (12, 13, 15, 17, 19). ETS exposure was reported to significantly increase BaP albumin adduct levels in one study (12), but not in other studies (13, 21). Exposure to PAH from ambient air (traffic exhaust) had no, or only a marginal, effect on the BaP adduct levels (18, 21). Elevated BaP-hemoglobin adduct levels were found in winter compared with summer (22).

The purpose of our investigation was to elucidate the influence of smoking, exposure to ETS, and diet on the exposure to PAH in healthy, nonoccupationally exposed subjects.

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[3] The abbreviations used are: PAH, polycyclic aromatic hydrocarbon; BaP, benzo(*a*)pyrene; 1-OHP, 1-hydroxypyrene; BPDE, benzo[*a*]pyrene diolepoxide; ETS, environmental tobacco smoke; LOD, limit of detection; GC-MS, gas chromatography-mass spectroscopy; HPLC, high-performance liquid chromatography; NICI, negative ion chemical ionization.

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The extent of exposure to PAH was determined by measuring the PAH biomarkers urinary 1-OHP six times per subject and the BaP adducts with albumin and hemoglobin once during the 8-month study period.

Materials and Methods

Subjects and Study Design. Sixty-nine subjects (41 men and 28 women), 18–70 years of age (mean ± SD, 35.4 ± 10.5 years), took part in the study, which lasted from March to October 1996. The subjects were selected so that the subgroups of "smokers," "ETS-exposed nonsmokers" (nonsmokers who have a smoking spouse or reported frequent ETS exposure), and "nonsmokers" (subjects who have a nonsmoking spouse and reported to be rarely exposed to ETS) were of about equal size. Ex-smokers who had quit smoking for >1 year were classified as nonsmokers. All subjects were healthy and lived in the city or suburbs of Munich, Germany. Written informed consent was obtained from all subjects before taking part in the study. The subjects visited the laboratory six times at intervals of 4–8 weeks. At each visit, a blood sample was taken and a 24 h-urine sample was returned. The urine was collected from the day before the visit until the morning of the visit. The subjects completed a protocol on their daily eating, drinking, and tobacco smoke exposure. The smokers recorded the number of cigarettes smoked during each hour. The nonsmokers indicated the times when they were exposed to ETS and were asked to rate the intensity in three grades (1 = low, 2 = medium, and 3 = strong exposure to ETS). This protocol covered the 7 days before each visit and was structured on an hourly basis. The nonsmokers were asked to wear a personal sampler for nicotine and 3-ethenylpyridine during the 7 days before the first three visits and during 5 days before the following three visits. All variables, except for the BaP protein adducts, were measured at each visit (six times), and the means were used for evaluation. The BaP adducts were only measured in the samples from the second visit.

Dietary BaP Intake. On the basis of published data (2, 23–29), the following BaP concentrations of food products were used for estimating the BaP intake from the reported food consumption data (μg/kg): cereals and bread, 0.2; leafy vegetables, 5.0; root vegetables and fruits, 0.2; meat and sausages, 3.0; fish, 1.0; and dairy products, 0.5.

Biological Samples. Twenty-four-h urine samples were collected after voiding the first morning urine until the following morning (including the first morning urine). The sample was stored in a cooling box until it was returned to the laboratory. In the laboratory, pH and volume were determined within 2 h, before the sample was divided into several fractions and frozen at −20°C.

Blood samples were drawn into EDTA-tubes and separated by centrifugation (500 × *g*, 10 min, 10°C) into plasma and erythrocytes. The blood fractions were stored until analysis at −20°C.

Analytical Methods

Albumin adducts of BaP. The method has been described elsewhere (30). Briefly, an equivalent of 100–200 mg of albumin (corresponding to 2–5 ml of plasma), together with 330 pg of (±)-BaP-r-7,t-8,c-9,c-10-tetrahydrotetrol (BT II-2; NCI Carcinogen Standard Repository, Midwest Research Institute, Kansas City, MO) as internal standard, was digested overnight with 20 mg of Pronase E (*Streptomyces griseus*, 7000 units/g; Boehringer Mannheim, Mannheim, Germany) to destroy the tertiary structure of the protein. The BaP-related esters were cleaved under vacuum conditions at pH 11 and 80°C for 2 h. After solid phase extraction with C18-cartridges, the sample was applied to an RP18-Alkyl-Diol-Silica enrichment column (RP18-ADS; Merck, Darmstadt, Germany) connected to a HPLC system (Model 1050; Hewlett Packard, Waldbronn, Germany). The fraction of the eluate containing the analyte (±)-BaP-r-7,t-8,t-9,c-10-tetrahydrotetrol (BT I-1) and the internal standard BT II-2 was collected, and the methanol was evaporated, diluted with 4 ml of water, and extracted twice with 4 ml of ethyl acetate. (±)-BaP-r-7,t-8,t-9,t-10-tetrahydrotetrol (BT I-2, 220 pg.; NCI Carcinogen Standard Repository, Midwest Research Institute) was added as a second internal standard. After silylation, 10 μl were injected into the GC-MS system (GC 8000 Top and MD 800 from ThermoQuest, Egelsbach, Germany). NICI was applied using methane as a reactant gas. For quantitation of the results, the ion m/z 284 (relative abundance, 46%) was used while the fragment ions at m/z 446.1 and m/z 447.1 (relative abundances, 19% and 18%, respectively) served for confirmation. Adduct concentrations were calculated by a five-point calibration curve in the range of 0.04- 4.0 pg of BT I-1/injection. BT I-1 for calibration was obtained from the NCI Carcinogen Standard Repository (Midwest Research Institute). The LOD for the albumin adducts of BaP was 0.011 fmol/mg, and the recovery rate was 51 ± 26% (n = 69). All 69 samples from the second visit were run in duplicate, with a coefficient of variation of 22%.

Hemoglobin Adducts of BaP. Washed erythrocytes from 10 ml of blood were lysed with 20 ml of bidestilled water and centrifuged (13,000 × *g*, 45 min, 4°C). The hemoglobin concentration in the supernatant was determined spectrophotometrically (31). After adding the internal standard (BT II-2) to 200 mg of hemoglobin, the protein was digested with Pronase (16 h, 37°C) to destroy the tertiary structure and the adducts were hydrolyzed (pH 11, 2 h, 80°C). The aqueous hydrolysate was applied to an Extrelut 20-column (1.5 × 23 cm, 80 ml; Merck) and eluted with 40 ml of ethyl acetate. The solvent was removed with a rotary evaporator and finally in a nitrogen stream. The dry residue was dissolved in 1 ml of water/methanol (80:20, v/v) and injected into the HPLC. The further steps were performed as described for the albumin adducts of BaP (see above and Ref. 30). The LOD for the hemoglobin adducts of BaP was 0.007 fmol/mg, and the recovery rate was 55 ± 17% (n = 69). All 69 samples from the second visit were run in duplicate, with a coefficient of variation of 30%.

Urinary 1-OHP. 1-OHP was determined according to a published method (32). Briefly, to 10 ml of urine, 20 ml of 0.1 M acetate buffer (pH 5.0) and 13 μl of β-glucuronidase/arylsulfatase (Boehringer Mannheim) were added and incubated for 5 h at 37°C in the dark. The solution was applied to a Sepak C18 cartridge (Varian GmbH, Darmstadt, Germany) and eluted with 2 ml of methanol. After the addition of 2 ml of 20 mM potassium dihydrogenphosphate, 20 μl were injected into the HPLC system (Model 2510 pump system, Model 9090 autosampler, and Model 4270 integrator; Varian GmbH), equipped with a column oven and a fluorescence detector (Model FP-920; Jasco, Groß-Umstadt, Germany). A C18-guard column and a C18-analytical column (4.6 × 250 mm; Symmetry, Waters, Milford, MA) were used. The retention time for 1-OHP was 19.5 min. The calibration curve (10 points) was constructed with spiked urine samples (0.05- 0.5 μg/l). The recovery rate was 94%, and the LOD was 0.01 μg/l. All urine samples (69 subjects × 6 time points − 5 missing samples =

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Table 1 Some characteristics and self-reported, as well as objectively, measured exposure data of the study groups

	Means (SE)			
	All nonsmokers	Nonsmokers	Passive smokers	Smokers
Number	42	23	19	27
Sex (% female)	50	48	53	26[a,b]
Age (yr)	37.6 (1.7)	40.8 (2.3)	33.8 (2.3)[a,c]	32.0 (1.7)[a,b]
Cigarette consumption (cig/d)[d]	0	0	0	17.7 (2.1)
ETS exposure duration (h/day)[d]	1.37 (0.29)	0.52 (0.10)	2.43 (0.56)[c,e]	
ETS exposure intensity[d,f]	1.86 (0.36)	0.67 (0.11)	3.31 (0.67)[c,g]	
Dietary BaP intake (μg/day)[h]	0.56 (0.03)	0.60 (0.05)	0.50 (0.04)	0.55 (0.04)
Nicotine on personal samplers ($\mu g/m^3$)[d]	0.431 (0.086)	0.185 (0.041)	0.730 (0.161)[c,e]	
3-Ethenylpyridine on personal samplers ($\mu g/m^3$)[d]	0.077 (0.011)	0.041 (0.005)	0.121 (0.020)[c,g]	
Thiocyanate in plasma (μmol/l)[d]	20.9 (2.0)	22.0 (2.8)	19.6 (3.1)	86.7 (8.4)[b,g]
Cotinine in plasma (ng/ml)[d]	0.98 (0.12)	0.71 (0.07)	1.32 (0.24)[a,c]	298 (39)[b,g]
Cotinine in urine (μg/24 h)[d]	6.8 (2.5)	2.3 (0.5)	12.3 (5.4)[a,c]	2060 (300)[b,g]

[a] $P < 0.05$. Statistical significance was determined with the t test for independent samples.
[b] Comparison between smokers and all nonsmokers.
[c] Comparison between passive smokers and nonsmokers not or rarely exposed to ETS.
[d] For each subject (except for one smoker; see text), the mean of six investigations was used to calculate the overall mean.
[e] $P < 0.01$. Statistical significance was determined with the t test for independent samples.
[f] The ETS exposure intensity was calculated by multiplying the exposure duration with the reported strength of the exposure: 1, low; 2, medium; 3, strong.
[g] $P < 0.001$. Statistical significance was determined with the t test for independent samples.
[h] The dietary protocols for the 7 days before the second visit to the laboratory were evaluated as described in "Materials and Methods."

409) were run in duplicate, with a coefficient of variation of 8%.

Cotinine in Plasma. Cotinine in plasma was determined by a RIA (33, 34). The LOD was 1 μg/l. All 409 plasma samples were run in duplicate, with a coefficient of variation of 10%.

Cotinine in Urine. Cotinine in urine was determined by GC with a nitrogen-selective detector according to a published method (35). The LOD was 1 μg/l. About 40% of the 409 urine samples were run in duplicate, with a coefficient of variation of 5%.

Thiocyanate in Plasma. Thiocyanate in plasma was determined photometrically according to a published method (36). The method was adapted to microtiter plate scale. The difference of the absorbance after adding iron(III)nitrate (formation of a *red*-colored complex) and after the addition of mercury-(II)nitrate (formation of a colorless complex) was measured at a wavelength of 492 nm.

Nicotine and 3-Ethenylpyridine on Personal Samplers. Nicotine and 3-ethenylpyridine were sampled on passive diffusion samplers (37) worn by the nonsmoking subjects over 5 or 7 days and analyzed as reported (37). The LOD was 0.01 $\mu g/m^3$ for both compounds using GC with nitrogen-selective detection.

Statistical Methods. Differences between the three groups according to smoking status (nonsmokers, passive smokers, smokers) were tested with the Students t test. The associations between the PAH biomarkers (dependent variables) and urinary cotinine or BaP intake with the diet (independent variables) were analyzed by linear regression. All calculations and tests were performed with the SPSS 8.0 software package (SPSS ASC GmbH, Erkrath, Germany).

Results

Characteristics of the Subgroups According to Smoking Status. Some characteristics of the three subgroups, including demographic data, self-reported duration and extent of exposure to ETS, cigarette consumption, reported dietary intake of BaP, and objective measurements on tobacco smoke exposure are presented in Table 1. Three of the 45 self-reported nonsmokers had consistently (at least at five of the six visits to the laboratory) elevated cotinine levels in plasma (>10 ng/ml) and in urine (>100 μg/24 h). These subjects were all male and reported to be exposed to ETS at home and/or at the workplace. The cotinine levels (mean ± SD, range for the six visits) were: subject 36, 67.5 ± 37.7, 20–120 ng/ml (plasma), 602 ± 367, 50–1073 μg/24 h (urine); subject 62, 63.2 ± 33.5, 1–110 ng/ml (plasma), 252 ± 143, 1–384 μg/24 h (urine); and subject 69, 124.5 ± 119.5, 15–374 ng/ml (plasma), 246 ± 175, 43–594 μg/24 h (urine). Because these cotinine concentrations were compatible with occasional or regular smoking (38–40), these subjects were reclassified as smokers. One subject (no. 26) was admitted to the study as a smoker but stopped smoking after the second visit to the laboratory. The subject was classified as smoker, but only the data of the first two visits were included in the evaluation.

The estimated average BaP intake with the diet ranged from 0.50–0.60 μg/day and was not significantly different between the various study groups.

Exposure to Tobacco Smoke. As expected, the biomarkers of tobacco smoke exposure (thiocyanate in plasma, cotinine in plasma and urine) are significantly elevated in smokers compared with nonsmokers. The ratio (smokers:all nonsmokers) is about 300 for cotinine in plasma or urine and about 4 for thiocyanate in plasma.

Nonsmokers classified as ETS exposed (passive smokers) reported significantly longer ETS exposure durations (2.43 *versus* 0.52 h/day) and significantly higher ETS exposure intensities (3.31 *versus* 0.67) compared with the nonexposed or only marginally ETS-exposed nonsmokers. The personal samplers of the passive smokers showed higher exposure to nicotine (0.730 *versus* 0.184 $\mu g/m^3$) and 3-ethenylpyridine (0.121 *versus* 0.041 $\mu g/m^3$) than those of the unexposed nonsmokers. Cotinine levels in plasma (1.32 *versus* 0.71 ng/ml) and urine (12.3 *versus* 2.3 μg/24 h) were also significantly higher in passive smokers than in unexposed nonsmokers. There was no significant difference in the plasma thiocyanate levels between these two groups (19.6 *versus* 22.0 μmol/l).

PAH Biomarkers in Relation to Tobacco Smoke Exposure. In Table 2, the means, SEs, and the ranges of the PAH biomar-

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Table 2 Levels of the PAH biomarkers urinary 1-OHP, BaP-hemoglobin adducts, and BaP-albumin adducts in the study groups

	Means[a] (SE) [range]			
	All nonsmokers	Nonsmokers	Passive smokers	Smokers
Number	42	23	19	27
Urinary 1-OHP (μg/24 h)[b]	0.157 (0.013) [0.05–0.46]	0.171 (0.020) [0.06–0.46]	0.140 (0.014) [0.05–0.26]	0.346 (0.048) [0.11–1.16][c,d]
BaP-hemoglobin adducts (fmol/mg)[e]	0.068 (0.014) [ND[f]–0.49]	0.083 (0.024) [ND–0.49]	0.049 (0.007) [ND–0.09]	0.105 (0.020) [0.01–0.44]
Number of samples (%) below the LOD of 0.007 fmol/mg	1 (2.4)	0 (0)	1 (5.3)	0 (0)
BaP-albumin adducts (fmol/mg)[e]	0.020 (0.005) [ND–0.192]	0.019 (0.008) [ND–0.192]	0.021 (0.007) [ND–0.101]	0.042 (0.011)[d,g] [ND–0.254]
Number of samples (%) below the LOD of 0.01 fmol/mg	29 (69.0)	16 (69.6)	13 (68.4)	13 (48.1)

[a] For samples with levels below the LOD, ½ LOD was used for the calculation of the means.
[b] For each subject (except for one smoker; see text), the mean of six investigations was used to calculate the overall mean.
[c] $P < 0.001$. Statistical significance determined with the t test for independent samples.
[d] Comparison between smokers and all nonsmokers. There were no significant differences between passive smokers and nonsmokers not or rarely exposed to ETS.
[e] The blood samples for the adduct determinations were drawn on the second visit of the subjects to the laboratory.
[f] ND, not detectable (<LOD).
[g] $P < 0.05$. Statistical significance determined with the t test for independent samples.

kers urinary 1-OHP, BaP-hemoglobin adducts, and BaP-albumin adducts for the various study groups are shown. Smokers excreted significantly higher amounts of 1-OHP than nonsmokers (0.346 *versus* 0.157 μg/24 h). The determination of BaP-hemoglobin adducts revealed one sample (a nonsmoker) that was below the LOD of 0.007 fmol/mg, whereas for the BaP-albumine adduct measurements, 13 samples of the 27 smokers (48.1%) and 29 samples of the 42 nonsmokers (69.0%) were below the LOD of 0.01 fmol/mg. Within the group of nonsmokers, the percentage of undetectable samples for BaP-albumin adducts was 16 of the 23 unexposed nonsmokers (69.6%) and 13 of the 19 passive smokers (68.4%). The means of the BaP-albumin adducts was significantly elevated in smokers compared with nonsmokers (0.042 *versus* 0.020 fmol/mg), whereas the BaP-hemoglobin adducts tended to be higher in smokers compared with nonsmokers (0.105 *versus* 0.068 fmol/mg; not significant). No significant differences between passive smokers and unexposed nonsmokers were observed for urinary 1-OHP (0.140 *versus* 0.171 μg/24 h), BaP-hemoglobin adducts (0.049 *versus* 0.083 fmol/mg), and BaP-albumin adducts (0.021 *versus* 0.019 fmol/mg).

For smokers, a significant correlation between the urinary excretion of cotinine and 1-OHP in urine ($r = 0.76$) and the BaP-albumin adduct levels ($r = 0.44$) was found (Fig. 1). The correlation between urinary cotinine and the BaP-hemoglobin adduct levels was not significant ($r = 0.19$). In nonsmokers, urinary cotinine was not correlated to 1-OHP ($r = -0.09$), BaP-hemoglobin adducts ($r = -0.15$), and BaP-albumin adducts ($r = 0.20$; Fig. 2).

PAH Biomarkers in Relation to Dietary BaP Intake. The estimated BaP intake from the diet of all subjects ($n = 69$) did not correlate with the urinary 1-OHP excretion ($r = 0.06$) or the BaP adducts of hemoglobin ($r = 0.05$) and albumin ($r = -0.01$). Similarly, nonsignificant correlations were found when the dietary BaP intake was standardized for the body weight or when the nonsmokers ($n = 42$) and smokers ($n = 27$) were analyzed separately.

PAH Biomarkers in Relation to Urban or Suburban Residence. The subjects were classified as "urban" or "suburban" according to the postal code of their home address. Urban residence means that the subject lives in the city of Munich, whereas suburban residence was within a radius of 15–60 km of the city center. In Fig. 3, the PAH biomarkers for all subjects divided into urban and suburban groups are shown. The significant effect of smoking on all three biomarkers is visible. Furthermore, the BaP adduct levels with hemoglobin and albumin tended to be higher in subjects with suburban residences. This effect reached borderline significance ($P = 0.056$) for BaP-albumin adducts of all subjects. No difference in urinary 1-OHP excretion was observed between subjects residing in the suburb or city (Fig. 3).

Discussion

Except for certain workplaces, exposure to PAH by dietary intake, smoking, breathing of ambient air, and passive smoking are regarded as most relevant for the general population (24). We determined three PAH biomarkers to quantify the exposure in a longitudinal field study with 69 subjects not occupationally exposed to PAH. In particular, we investigated the role of exposure to tobacco smoke (both active and passive smoking) on the PAH biomarkers. Furthermore, we elucidated the influence of PAH intake with the diet assessed by daily protocols and published PAH contents of major food items. Finally, the influence of the area of residence (urban or suburban) on the overall PAH burden was investigated.

Smoking. The mainstream smoke of a filter cigarette contains about 10 ng of BaP (3, 4). Smoking, on average, 18 cigarettes/day, as the smokers in this study did (Table 1), would result in a smoking-related BaP intake of 180 ng/day. We found 2-fold increased PAH biomarker levels in smokers compared with nonsmokers (Table 2). The effect of smoking was significant for urinary 1-OHP ($P < 0.001$) and BaP-albumin adducts ($P < 0.05$), but did not reach statistical significance in the case of BaP-hemoglobin adducts ($P = 0.12$). Furthermore, we observed a significant correlation between the extent of smoking (measured as the urinary excretion of cotinine) and urinary 1-OHP ($r = 0.76$; $P < 0.01$) and BaP-albumin adducts ($r = 0.44$; $P < 0.05$; Fig. 1). There was no significant correlation between urinary cotinine and BaP-hemoglobin adducts ($r = 0.19$, $P = 0.35$). These findings confirm other reports on the effect of smoking on PAH biomarkers. Van Rooij *et al.* (5) reported urinary excretion of 1-OHP of 0.09–0.13 μmol/mol creatinine for nonsmokers and 0.24–0.34 μmol/mol creatinine for smokers. These levels are higher than in our study when converted to daily excretion rates, assuming a creatinine excretion of 1.5 g/day (nonsmokers, 0.26–0.38 μg/24 h; smokers, 0.69–0.98 μg/24 h). Van Rooij *et al.* (5) reported a correlation

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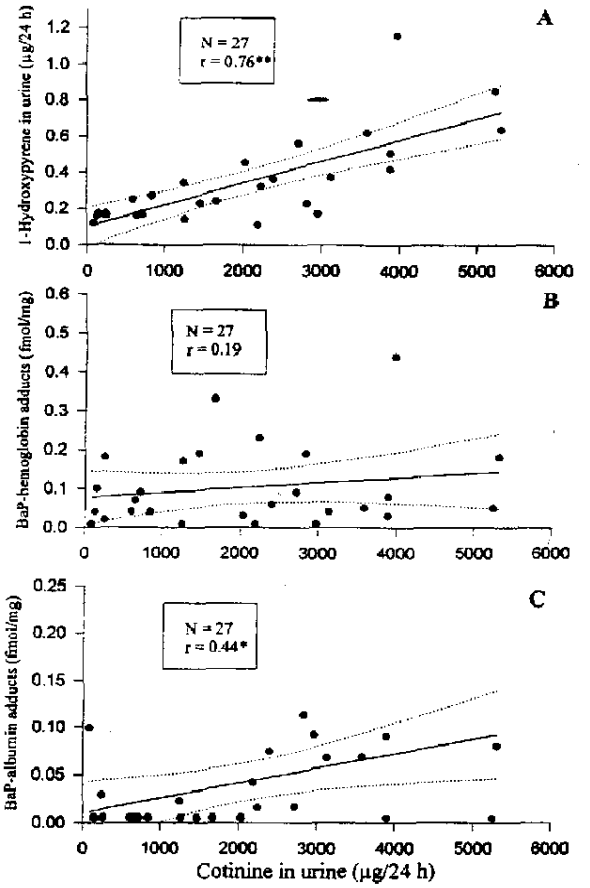

Fig. 1. Linear regression between urinary cotinine and urinary 1-hydroxypyrene (*A*), BaP-hemoglobin adducts (*B*), and BaP-albumin adducts (*C*) in smokers. The *solid line* is the regression line, and the *broken line* is the 95% confidence interval. Levels of significance: *, $P < 0.05$; **, $P < 0.01$.

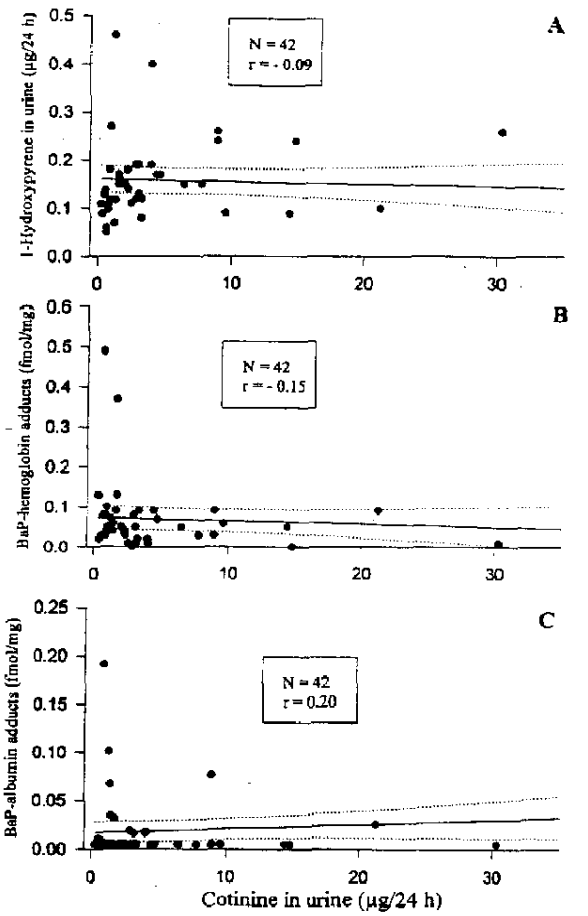

Fig. 2. Linear regression between urinary cotinine and urinary 1-hydroxypyrene (*A*), BaP-hemoglobin adducts (*B*), and BaP-albumin adducts (*C*) in nonsmokers. The *solid line* is the regression line, and the *broken line* is the 95% confidence interval.

coefficient between urinary 1-OHP excretion and the average number of cigarettes per day of $r = 0.67$ ($P = 0.001$), which is in good agreement with our reported correlation with urinary cotinine ($r = 0.76$; Fig. 1) and with daily cigarette consumption ($r = 0.59$, $P < 0.01$). In another study (41), the difference in 1-OHP excretion between occupationally exposed smokers and nonsmokers (foundry workers) was insignificant, whereas the difference in the not occupationally exposed control group was of borderline significance ($P = 0.06$). Pastorelli *et al.* (22) reported two times higher levels of urinary 1-OHP in smokers compared with nonsmokers. Sithisarankul *et al.* (42) reported a concentration of 1-OHP-glucuronide of 1.04 pmol/ml for smokers and 0.55 pmol/ml for nonsmokers ($P = 0.001$), a ratio similar to that in our study. The correlation with daily cigarette consumption was weaker than in our study ($r = 0.34$)

Melikian *et al.* (19) observed significantly higher BaP-hemoglobin adducts in 10 smokers (mean, 2.6; range, 1.2–7.8 fmol/mg) than in 10 nonsmokers (mean, 0.97; range, 0.7–1.3 fmol/mg). It is noteworthy that the BaP adduct levels in our study were at least one order of magnitude lower. In a study with 44 smokers (lung cancer patients), Pastorelli *et al.* (16) found detectable levels of BaP-hemoglobin adducts (LOD, 0.05 fmol/mg) in only six subjects (13.6%). The median for all 44 samples was 0.025 fmol/mg (half the detection level). In our study, comprising subjects with low to moderate traffic exposure, only one subject had a BaP-hemoglobin level below the detection level of 0.007 fmol/mg (Table 2). The median was 0.064 fmol/mg for smokers and 0.046 fmol/mg for nonsmokers, comparable with the values reported by Pastorelli *et al.* (16). In an earlier study, Pastorelli *et al.* (18) reported median BaP-hemoglobin adduct levels of ≤0.1 fmol/mg and ≤0.26 fmol/mg for newspaper vendors with low and extensive traffic exposure, respectively. The difference was not significant. In a recent publication (22), the same group reported significantly lower adduct levels in summer (mean, 0.031 fmol/mg) than in winter (mean, 0.14 fmol/mg). No effect of smoking and diet was found.

The smokers in our study had significantly elevated BaP-albumin adduct levels compared with the nonsmokers (Table 2). Thirteen samples (48.1%) from the smokers and 29 samples (69.0%) of the nonsmokers were below the LOD (0.01 fmol/

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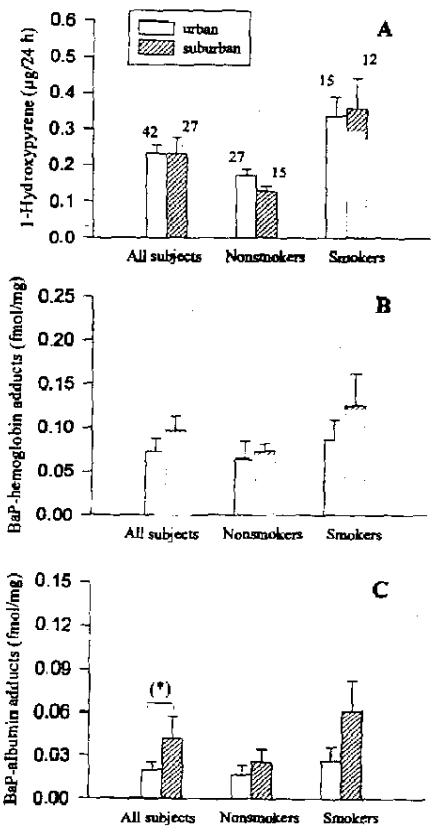

Fig. 3. Levels of urinary 1-hydroxypyrene (*A*), BaP-hemoglobin adducts (*B*), and BaP-albumin adducts (*C*) for all subjects ($n = 69$), nonsmokers ($n = 42$), and smokers ($n = 27$) classified according to their location of residence (urban or suburban). The *bars* represent the means, and the *whiskers* represent the SE of the means. *, $P = 0.056$.

mg). The percentage of undetectable samples for unexposed and ETS-exposed nonsmokers was similar (69.6 and 68.4%, respectively; Table 2). Elevated BaP-albumin adduct levels in smokers have also been reported in other studies (12, 15, 17, 43). However, adduct levels in smokers/nonsmokers determined by ELISA were several orders of magnitude higher than in our investigation (Table 2): 14.2/7.23 fM BaP equivalents/100 μg (15), 0.80/0.41 fmol/μg (12), 5.78/4.67 fmol/μg (medians; Ref. 13). It can only be assumed that the antibodies used in the ELISA are selective for PAH rather than specific for BaP. Using HPLC with fluorescence detection, Tas *et al.* (17) found median BaP-albumin adduct levels of 1.62 fmol/mg for smokers and 1.39 fmol/mg for nonsmokers. Also, these levels were almost two orders of magnitude higher than those in our study, suggesting probably an unspecific detection of the BaP-tetrol. In the study of Pastorelli *et al.* (16), in which GC-MS-NICI was applied for adduct determination, 24 of 44 smokers (56.8%) had undetectable (LOD, 0.05 fmol/mg) BaP-albumin adduct levels. The median of all 44 samples was 0.11 fmol/mg, which is about 7-fold higher than the median for smokers in our study (0.015 fmol/mg). Part of the discrepancy could be due to more intense smoking in the Italian study group compared with ours.

Passive Smoking. Indoor air concentrations of BaP in smoking and nonsmoking homes are reported to be 1.0 ng/m^3 and 0.4 ng/m^3, respectively (44). In commercial buildings, mean BaP concentrations of 1.07 ng/m^3 and 0.39 ng/m^3 in smoking and nonsmoking environments, respectively, have been found (45). Therefore, the additional ETS-related BaP exposure is about 0.6 ng/m^3. Assuming a daily ETS exposure duration of about 3 h (Table 2; Refs. 46 and 47), a respiration rate of 1 m^3/h would result in a maximum intake of 1.8 ng BaP/day due to passive smoking, which accounts for about 1% of the overall daily BaP intake in nonsmokers. It is, therefore, not surprising that we found no influence of exposure to ETS on any of the three PAH biomarkers (Table 2 and Fig. 2).

The evidence from the BaP adduct measurement is limited due to the fact that for a major part of the samples the adduct levels were at or below the LOD (Table 2). On the other hand, the 1-OHP measurements, in our view, provide strong evidence because for each subject six independent samples were analyzed and the concentrations were well above the LOD in all samples.

We took great effort to determine the extent of exposure to ETS: (*a*) nonsmokers were selected either to be not or only marginally exposed to ETS or to be regularly exposed to ETS at home, at work, or in leisure time; (*b*) the subjects were asked to keep records of their ETS exposure in terms of duration and intensity during 6 weeks; (*c*) the nonsmokers also wore personal samplers for nicotine and 3-ethenylpyridine during the 6 weeks; and (*d*) cotinine in plasma and urine, suitable biomarkers for ETS exposure (48, 49), were measured on six different occasions. No association between plasma thiocyanate and passive smoking was observed (Table 2). This confirms an earlier report in the literature (50), suggesting that plasma thiocyanate is not a suitable biomarker for determining exposure to ETS. Friedman *et al.* (51) found that only about 2% of the variation in serum thiocyanate concentration is explained by passive smoking. There was satisfactory consistency between the other ETS exposure markers, both objectively measured and subjectively reported, except for plasma cotinine. The reason for this is probably the fact that for all nonsmokers the plasma cotinine concentration was at or below the LOD of 1 μg/l for at least one time point. Taken together, we are confident that our assessment of the extent of exposure to ETS is reliable and that there is no measurable effect of real-life passive smoking on urinary 1-OHP or on BaP adduct levels with hemoglobin or albumin.

Diet. The method of choice for determining the BaP intake from food seems to be the "duplicate meal" approach. Using this method, daily BaP intakes of 123 ng (24) and 176 ng (6) were found. We estimated a mean dietary BaP intake of 500–600 ng/day for the various subgroups in our study (Table 1), which is clearly higher than the values found with the duplicate meal method but corresponds with some of the reported ranges in the literature (8). A reason for the large variations of the BaP intake estimates is certainly the heterogenous array of cooking styles and foodstuffs used (24). With the limitations described above, it is comprehensible that we found no correlation of the estimated BaP intake with the diet and any of the PAH biomarkers.

In general, it can be stated that although diet is recognized as the most important source of PAH exposure for nonoccupationally exposed nonsmokers (5, 6, 52), only weak associations between the estimated PAH intake from the diet and various PAH biomarkers were found. We believe that the inaccuracy of the estimate for the PAH intake is the major reason for this observation.

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Ambient Air. We looked for a possible effect of urban or city residence on the concentrations of the PAH biomarkers. Unexpectedly, we observed a trend for higher BaP adduct levels with hemoglobin and albumin for subjects living in the suburbs, whereas the place of residence had no influence on the urinary excretion of 1-OHP (Fig. 3). The smoking behavior is not responsible for this result. Presently, we have no reasonable explanation for this finding.

Pastorelli *et al.* (18) found significantly increased BaP-hemoglobin adducts in traffic-exposed, nonsmoking newspaper vendors compared with their unexposed counterparts (median, 0.3 *versus* ≤ 0.1 fmol/mg). In smokers, no influence of exposure to traffic was observed (0.27 *versus* 0.26 fmol/mg).

Nielsen *et al.* (21) found elevated BaP-albumin adduct levels in subjects living in rural areas compared with urban areas of Denmark (4.54 *versus* 3.54 fmol/μg, determined by ELISA). The difference was not significant. Interestingly, significantly higher DNA adduct levels (determined by ^{32}P-postlabeling) in lymphocytes of the urban subjects were observed (21). Protein and DNA adducts did not correlate in this study. DNA adduct levels in lymphocytes were also found to increase from rural controls to bus drivers working in the dormitory, suburban and center of Copenhagen (53).

In a study with 130 pregnant women living in urban, suburban, and rural areas of the county of Aarhus, Denmark, Autrup and Vestergaard (43) found about similar levels of BaP-albumin adducts (determined with an ELISA) in subjects from urban and rural parts, whereas subjects living in the suburbs had significantly lower adduct levels. Because the samples were collected in November/December, the authors speculated that differences in domestic heating sources were responsible for these findings.

Conclusion. In conclusion, our results show that smoking increases the PAH exposure of nonoccuptionally exposed persons by a factor of two, whereas passive smoking does not measurably increase the overall PAH burden. We suggest that diet is, by far, the most important source for PAH intake in nonsmokers, although, probably due to the inaccuracy of the intake estimates, we were not able to find an association between the dietary PAH intakes and the levels of the PAH biomarkers. Our results further indicate that PAH exposure through ambient air might be of some importance. Part of the interindividual variation in the PAH biomarkers is certainly due to the genetic polymorphism in metabolic activation and detoxification pathways (for review see Ref. 54). The influence of the genetic polymorphism in this study is presently under investigation in our laboratory. Finally, the levels of the BaP-protein adducts that we measured are partly much lower than those reported by others also using GC-MS-NICI.

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